Biological Metal Clusters: Biophysical and Model Studies (1)

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Nickel K-edge XAS is being employed to examine the Ni-Fe heterodinuclear active site in various forms of hydrogenase (H_2ase) related by redox poise and inhibitor complexes. A manuscript detailing the structures of six redox-related forms, the photoproduct derived from the reduced epr-active state (Form C) and the CO inhibitor complex of *Chromatium vinosum* H_2ase is in press (Davidson, G., Choudhury, S.B., Gu, Z., Bose, K., Roseboom, W., Albracht, S.P.J., Maroney, M.J. *Biochemistry, 39*: 2000). This study presents the first structural details of the Ni sites for the kinetically 'ready' epr silent state (SI_r) and its CO complex (SI-CO) in any H_2ase. Analysis of Ni K-edge energy shifts in redox-related samples reveals that reductive activation is accompanied by an oscillation in the electron density of the Ni site involving formally Ni(III) and Ni(II), where all the epr-active states (Forms A, B, and C) are formally Ni(III) and the epr-silent states are formally Ni(II). Analysis of XANES shows that the Ni site undergoes changes in the coordination number and geometry that are consistent with five-coordinate Ni sites in forms A, B and SI_u; distorted four-coordinate sites in SI_r and R; and a six-coordinate site in Form C. EXAFS analysis reveals that the loss of a short Ni-O bond accounts for the change in coordination number from five to four that accompanies formation of SI_r and activation of the enzyme. A shortening of the Ni-Fe distance from 2.85 Å in Form B to 2.60 Å also occurs at the SI level and is thus associated with the loss of the bridging O-donor ligand in the active site. Multiple-scattering analysis of the EXAFS data for the SI-CO complex reveals the presence of Ni-CO ligation, where CO is bound in a linear fashion appropriate for a terminal ligand. The putative role of Form C in binding H_2 or H^- was examined by comparing the XAS data from Form C with that of its photoproduct, Form L. The data rule out the suggestion that the increase in charge density on the NiFe active site that accompanies the photo process results in a two-electron reduction of the Ni site [Ni(III) -> Ni(I)].

Work employing *Methanococcus voltae* and *Desulfomicrobium baculatum* H_2ases, enzymes that have a SeCys Ni ligand, to explore the role of the ligands in this chemistry are in progress.